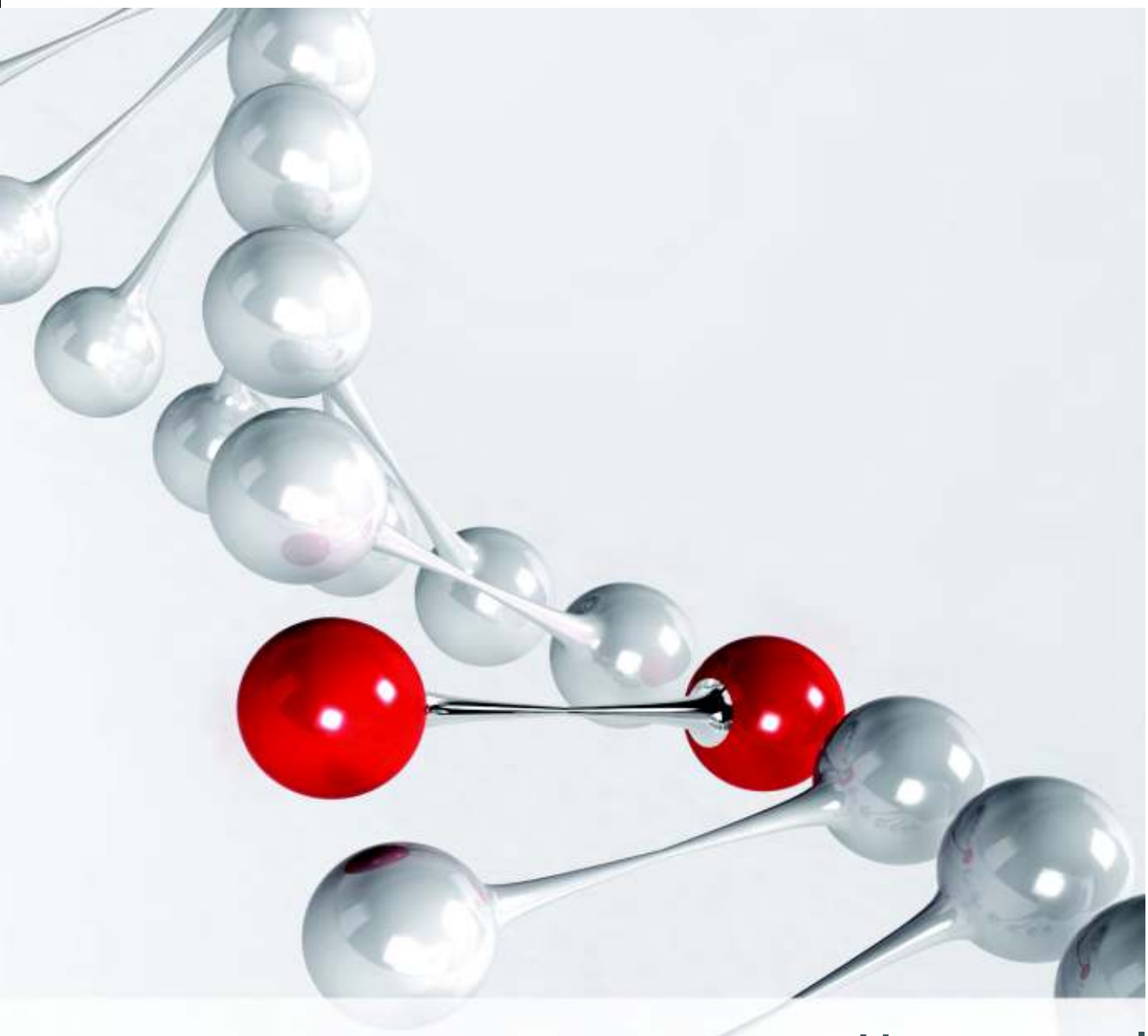
stratec
molecular



User manual
Invisorb® Spin Universal Kit

for simultaneous isolation of genomic and bacterial DNA, viral DNA and viral RNA from 200 µl of fresh or frozen plasma, serum, urine, cell free body fluids as well as rinsed liquid from swabs and supernatant from stool suspension or whole blood (100 µl).

CE

IVD

REF 1050100x00

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for Invisorb® Spin Universal Kit

The **Invisorb® Spin Universal Kit** is the ideal tool for isolation and purification of higly pure total nucleic acid, like human genomic DNA, bacterial DNA, viral DNA and RNA from 200 µl of fresh or frozen plasma, serum, urine, cell free body fluids as well as rinsed liquid from swabs, breast milk, pretreated sputum, BAL and supernatant from stool suspension or whole blood (100 µl), and additionally only for veterinary applications from allantoic fluid or rinse liquid from cloacal or tracheal swabs and from organ abrasions.

Due to the high purity, the isolated DNA/RNA is ready to use for a broad panel downstream applications or can be stored at -20°C/-80°C for subsequent use.

The kit is neither validated for the isolation of total RNA from cultured or isolated cells, from tissue samples, whole blood or urine, nor for genomic DNA from tissue, blood cards or dried blood stains.

CE **IVD** Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for *in-vitro* diagnostic use in countries where the EU Directive 98/79/EC on *in vitro* medical devices is not recognized.

Trademarks: Invisorb®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb® is a registered trademark of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

© 2014 STRATEC Molecular, all rights reserved.

Contents

Kit contents of the Invisorb® Spin Universal Kit	**3**
Symbols	**4**
Storage	**4**
Quality Control	**4**
Intended use	**5**
Product use limitation	**5**
Safety information	**6**
Product characteristics of the Invisorb® Spin Universal Kit	**7**
Principle and procedure	**8**
Yield and quality of genomic DNA/ RNA from blood	**8**
Yield and quality of viral DNA/ RNA	**8**
Important notes	**9**
Equipment and reagents to be supplied by user	**9**
Important indications	**10**
Sampling, storage and preparing of starting materials	**10**
Preparation of starting materials	**12**
Lysis procedures	**14**
Preparation of a master mix	**14**
Extraction control	**14**
Instructions	**15**
Scheme for the Isolation of genomic DNA from blood	**16**
***Protocol 1:* Isolation of genomic DNA from blood**	**17**
Scheme for the simultaneous isolation of pathogen DNA and RNA from all liquid samples and from tissue biopsies	**18**
***Protocol 2*: Simultaneous isolation of pathogen DNA and RNA from all liquid samples**	**19**
***Additional protocol:* Simultaneous isolation of nucleic acids from tissue biopsies**	**21**
Troubleshooting	**23**
Appendix	**24**
Ordering information	**26**

Kit contents of the Invisorb® Spin Universal Kit

	10 Preparations	50 Preparations	250 Preparations
Catalogue No.	1050100900	1050100200	1050100300
Lysis Buffer HLT	2 x 2 ml	15 ml	60 ml
Proteinase K	for 250 µl working solution	for 1.1 ml working solution	for 3 x 2 ml working solution
Carrier RNA	for 240 µl working solution	for 1.2 ml working solution	for 3 x 2 ml working solution
RNase Free Water	2 ml	2 x 2 ml	15 ml
Binding Solution (fill with 99.7% Isopropanol)	3 x 1 ml (ready to use)	empty bottle (final volume 15 ml)	empty bottle (final volume 80 ml)
Wash Buffer HLT	15 ml (ready to use)	30 ml (final Volume 50 ml)	105 ml (final Volume 175 ml)
Wash Buffer	15 ml (ready to use)	2 x 18 ml (final Volume 2 x 60 ml)	2 x 60 ml (final Volume 2 x 200 ml)
Elution Buffer M	2 x 2 ml	30 ml	120 ml
RTA Spin Filter Set	10	50	5 x 50
RTA Receiver Tubes	20	2 x 50	10 x 50
1.5 ml Receiver Tubes	10	50	5 x 50
2.0 ml Safe–Lock-Tubes	10	50	5 x 50
Manual	1	1	1
Initial steps	Resuspend lyophillized **Proteinase K** by addition of 250 µl **RNase free Water** to the vial and mix thoroughly until completely dissolving and store at -20°C! Resuspend lyophillized **Carrier RNA** by addition of 240 µl **RNase free Water** to the vial and mix thoroughly until completely dissolving!	Resuspend lyophillized **Proteinase K** by addition of 1.1 ml **RNase free Water** to the vial, mix thoroughly until completely dissolving and store at -20°C! Resuspend lyophillized **Carrier RNA** by addition of 1.2 ml **RNase free Water** to the vial and mix thoroughly until completely dissolving! Fill 15 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 20 ml of 99.7% **Isopropanol** to the bottle **Wash Buffer HLT.** Mix thoroughly and always keep the bottle firmly closed! Add 42 ml of 99.8% Ethanol to the bottle Wash Buffer. Mix thoroughly and always keep the bottle firmly closed!	Resuspend lyophillized **Proteinase K** by addition of 2 ml **RNase free Water** to the vial, mix thoroughly until completely dissolving and store at -20°C! Resuspend lyophillized **Carrier RNA** by addition of 2 ml **RNase free Water** to each vial, mix thoroughly until completely dissolving! Fill 80 ml 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle Add 70 ml of 99.7% **Isopropanol** to the bottle **Wash Buffer HLT.** Mix thoroughly and always keep the bottle firmly closed! Add 140 ml of 99.8% Ethanol to the bottles Wash Buffer. Mix thoroughly and always keep the bottle firmly closed!

Symbols

Lot number

Catalogue number

Date of manufacture

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Manufacturer

Storage

All buffers and kit contents of the **Invisorb Spin Universal Kit** should be stored at room temperature and are stable for at least 12 months.

Room temperature (RT) is defined as range from 15-30°C.

Proteinase K: Dissolved Proteinase K must be stored at –20 °C. Dividing the Proteinase K into aliquots and storage at –20 °C is recommended.

Carrier RNA: Lyophilized Carrier RNA can be stored at 2 - 8°C or at RT, but the recommendation for long time storage is –20°C. Dissolved Carrier RNA must be stored at -80°C, but repeated freezing and thawing will degrade the Carrier RNA and reduce the functionality of the kit. Therefore dividing Carrier RNA into aliquots and storage at –80°C is recommended.

Wash Buffers: Wash Buffer charged with isopropanol or ethanol should be stored at room temperature and should be appropriately sealed. If any precipitates are visible within the provided solutions, solve these precipitates by careful warming up to 30°C.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **Invisorb® Spin Universal Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **Invisorb® Spin Universal Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **Invisorb® Spin Universal Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:
from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907/ 2903

or contact your local distributor.

Intended use

The **Invisorb® Spin Universal Kit** is designed for extraction and purification of DNA/RNA from 200 µl sample material (for blood sample only 100 µl) using the RTA Spin Filter system with capped spin columns.

The nucleic acid isolation protocol is suitable for routinely preparation of DNA/RNA from fresh or frozen samples. For reproducible and high yields an appropriate sample storage is essential (see “Sampling and storage of the starting material”, page 8).

The **Invisorb® Spin Universal Kit** is the ideal tool for reliable and fast simultaneous isolation of high quality genomic, bacterial and viral DNA as well as viral RNA from fresh or frozen human or mammalian blood, serum, plasma, swabs, cerebrospinal fluid, cell culture supernatants, urine, and other cell free body fluids.

The kit can furthermore be used for the isolation of high quality genomic, bacterial and viral DNA as well as viral RNA from the same kind of samples, but coming from animals. The amount of blood, depends on the kind of animals.

Blood samples have to be stabilized with EDTA or citrate, not heparin.

For reproducible high yields an appropriate sample storage and quick operation under the rules for RNA and DNA operation is essential. The purified DNA and/or RNA is ready to use for subsequent downstream analysis.

The isolation protocols as well as all buffers are optimized to provide high yield and purity of the extracted nucleic acids. The procedure requires minimal interaction by the user, allowing safe handling of potentially infectious samples.

Any diagnostic results generated using the sample preparation procedure in conjunction with any downstream diagnostic assays should be interpreted with regard to other clinical or laboratory findings.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings. To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The isolation of eukaryotic or total RNA from any kind of sample was not evaluated. The isolation of DNA/RNA from sample sources like fungi was neither tested nor validated.

Differing the starting material or flow trace may lead to inoperability. Therefore neither a warranty nor a guarantee in this case will be given, implied or expressed.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide validations of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory. The laboratory must be validated pursuant to CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes than intended.

The included chemicals are only useable once and are not suitable for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.stratec.com for each STRATEC Molecular Product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

Stratec Molecular has not tested the waste generated by the **Invisorb® Spin Universal Kit** procedures for residual infectious materials. Contamination of the waste with residual infectious materials is unlikely, but cannot be excluded completely. Therefore, the waste has to be considered infectious and should be handled and discarded according to local safety regulations.

European Community risk and safety phrases for the components of the **Invisorb® Spin Universal Kit** to which they apply are listed below as follows:

Lysis Buffer HLT, Wash Buffer HLT

warning

contains guanidine-hydrochloride;

H302-315-319, P280-305+351+338

Proteinase K

danger

H315-319-334-335 P280-305-351-338-310-405

H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses and continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
inside of USA: 1 – 800 – 535 – 5053

Product characteristics of the Invisorb® Spin Universal Kit

The **Invisorb® Spin Universal Kit** provides a fast and efficient way for reliable simultaneous isolation of high quality viral DNA / RNA, bacterial and human DNA from a diverse range of starting material. The procedures are suitable for use with blood, plasma or serum; either can contain citrate or EDTA (no heparin) urine, swabs and water. Samples can be fresh or frozen, they should not be frozen and thawed more than once.

Starting Material	Yield	Time for preparation
100 µl fresh or frozen blood, 200 µl fresh or frozen plasma/ serum, urine, CSF, supernatant from stool suspensions 200 µl rinsed liquid of swabs, pretreated sputum, BAL, breast milk <u>Additional materials only for veterinarian applications:</u> 20 - 100 µl animal blood 200 µl amniotic fluid 200 µl supernatant from organ abrasions	depending on sample (storage and source)	about 60 min per extraction

The amount of purified DNA and/ or RNA in the **Invisorb® Spin Universal Kit** procedures depend on the sample type, sample source, transport, storage, age and the virus titer. The procedure is designed to avoid sample-to-sample cross-contaminations and allow safe handling of potentially infectious samples.

The DNA/RNA isolation process is based on the interaction of nucleic acids with silica membranes in optimal buffer conditions.

After a sample specific lysis using **Lysis Buffer HLT** and **Proteinase K**, optimal binding conditions are adjusted by the addition of **Isopropanol.** The genomic DNA/RNA binds to the RTA Spin Filter. Subsequent to three washing steps of the membrane bound nucleic acids, the nucleic acids are finally eluted in **Elution Buffer M**.

Yield and quality of the isolated nucleic acids are suitable for a wide range of molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturer specifications. Due to the high purity, the isolated DNA/ RNA is ready to use for a broad panel of downstream applications or can be stored at –80°C for subsequent use.

- (RT)-PCR*
- real-time PCR (quantitative (RT)-PCR, like TaqMan und LightCycler technology)
- cDNA synthesis
- microarray application

Note: *Systems isolating simultaneously DNA and RNA using buffers adapted for the binding of DNA and RNA, but the optimal binding conditions of RNA and DNA are different, so that such solutions can show a little reduction in sensitivity in comparison to kits optimized to one kind of nucleic acid isolation.*

For further information please contact: +49 (0) 30 9489 2901, -2910 in Germany and from foreign countries +49 (0) 30 9489 2907, -2903 or your local distributor (see page 28).

Principle and procedure

The **Invisorb® Spin Universal Kit** procedure comprises following steps:

- Lysis and protein digestion
- Binding of the DNA/RNA to the filter membrane
- Washing the filter bound DNA/RNA and elimination of alcohol
- Elution of DNA/RNA

After lysis the DNA/RNA binds to the filter, contaminations and enzyme inhibitors are efficiently removed during the following wash steps and purified DNA/RNA is eluted in Elution Buffer M.

Lysis
Samples are lysed at elevated temperatures in the presence of **Lysis Buffer HLT** and **Proteinase K**.

For bacteria we recommend a pretreatment with Lysozyme at 37°C before lysis.

Binding nucleic acids
After adding **Isopropanol** to adjust optimal binding conditions the lysate will be applied onto the RTA Spin Filter and the nucleic acids are bound to the surface of the Filter membrane as the lysate is drawn through by centrifugation.

Removing residual contaminants
Contaminants are efficiently washed away using **Wash Buffer HLT** and **Wash Buffer**, while the nucleic acids remain bound to the membrane of the RTA Spin Filter.

Elution
High quality viral DNA/ RNA and genomic DNA is eluted from the membrane using **Elution Buffer M**. Eluting twice each time with 100 µl leads to a little increase of DNA/RNA yield. Usage of small elution volumes may raise the DNA/RNA concentration. Elution volumes should be at least 40 µl of Elution Buffer M. The volume of eluate recovered may be up to 5 µl less than the volume of elution buffer applied to the RTA Spin Filter. The volume of eluate recovered depends on the nature of the sample and the amount of Elution Buffer M used.
The eluted DNA/ RNA are ready to use in different subsequent applications.

Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipette tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipette tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard gloves if they become contaminated.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow.
- This kit should only be used by trained personnel.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information please consult the appropriate material safety data sheets (MSDS). (See our webpage: www.stratec.com)

1. Microcentrifuge ≥ 11.000 x g (≥ 11.000 rpm)
2. Thermomixer (37°C - 95°C)
3. Isopropanol (99.7%)
4. Ethanol (96-100%)
5. 2.0 ml reaction tubes (optional)
6. Measuring cylinder (250 ml)
7. Disposable gloves
8. Pipet with tips
9. 1.5 ml reaction tubes
10. optional centrifuge for 15 or 50 ml
11. optional Lysozyme (10 mg/ml)
12. optional PBS

*The **Invisorb® Spin Universal Kit** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth.**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Possible suppliers for centrifuges:

Eppendorf AG
22331 Hamburg, Germany
Tel.: +49 (0) 40 53801 0
Fax: +49 (0) 40 53801 556
E-Mail: eppendorf@eppendorf.com
Internet: www.eppendorf.com

SIGMA Laborzentrifugen GmbH
37507 Osterode am Harz, Germany
Tel.: +49-5522-5007-0
Fax: +49-5522-5007-12
E-Mail: info@sigma-zentrifugen.de
Internet: www.sigma-zentrifugen.de

Important indications

Carrier RNA

Carrier RNA serves the following purposes. It enhances the reversible binding of viral acids to the RTA Spin Filter membrane. The addition of Carrier RNA reduces the chance of viral nucleic acid degradation. It minimizes the binding of viral acid to the reaction tubes.

Handling of RTA Spin Filter

Do to the sensitivity of viral DNA/ RNA amplification technologies the following precautions are necessary when handling the RTA Spin Filter to avoid cross-contamination between sample preparation.

1. carefully apply the sample or solution to the RTA Spin Filter, pipet the sample onto the filter without wetting the rim of the column
2. always change pipet tips between liquid transfers, we recommend the use of aerosol barrier pipet tips
3. avoid touching the RTA Spin Filter membrane with the pipet tip

Yield and quality of genomic DNA/ RNA from Blood

The amount of purified DNA/RNA in the **Invisorb® Spin Universal Kit** procedure from whole blood depends on the leucocytes content, the sample source, transport, storage, and age.

The typical yield usually expected from the **Invisorb® Spin Universal Kit** is about 1 µg DNA.

Please keep in mind, that a small amount of CarrierRNA in the eluate will elevate the real genomic DNA content.

Yield and quality of pathogen DNA/ RNA

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates derived by this kit will contain Carrier-RNA, which will greatly exceed the amount of the isolated NA.

Yields of viral nucleic acids isolated from biological samples are usually low concentrated and therefore almost impossible to determine photometrically.*

*. Keep in mind that the Carrier-RNA (5 µg per 200 µl sample) will account for most of the present NA.

The kit is suitable for downstream analysis with NAT techniques, for examples qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed according to the manufacturer's instructions.

Quantitative RT-PCR is recommended for determination of viral RNA yield.

***** In Gel Electrophoresis and in Capillary Electrophoresis, DNA extracted with the provided kit looks like degraded cause the kit contains Carrier RNA, this is poly A RNA in fragments of 100 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.

Sampling, storage and preparing of starting materials

Please read the instructions carefully and conduct the prepared procedure.

Sampling and storage

For reproducible and high yields appropriate sample storage is essential. Yields may vary from sample to sample depending on factors such as health of the donor, sample age, kind of sample, transport and storage conditions.

Blood: Best results are obtained using fresh blood samples. Blood samples (stabilized with EDTA or citrate but not heparin) can be stored at room temperature (18-25°C) for 2-3 hours. For short time storage (up to 24 h) samples should be stored at 4-8°C. For long term storage, we recommend to freeze the samples at -20°C or -80°C. Avoid multiple thawing and freezing cycles of the sample(s) before isolating the DNA/ RNA.

Serum and plasma: After collection and centrifugation, serum, plasma, from blood (treated with anticoagulants like EDTA or citrate, but not with heparin), synovial fluid samples or other cell free body fluids, swabs as well as stool samples can be stored on ice for 1 - 2 hours, for short time (up to 24 h) samples may be stored at 20°C. For long term storage, we recommend freezing samples in aliquots at –80°C. Frozen plasma or serum samples must not be thawed more than once. Multiple thawing and freezing before isolating the viral DNA/ RNA should be avoided. It leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. In addition, cryoprecipitate formed during freeze-thawing could make problems. If cryoprecipitate is visible, they should be pelleted by centrifugation at app. 6.800 x g for 3 minutes. The cleared supernatant should be aspirated, without disturbing the pellet and processed immediately. This step will not reduce viral titers.

Swabs, saliva: The protocol works with fresh saliva, prepared swabs as well as with dried swabs. The protocol has not been validated for isolation of DNA from swabs which are stored under special storage buffers of other provider.

Best results are obtained using freshly extracted samples. As long as the samples are not shock frosted with liquid nitrogen or are incubated with RNase inhibitors or denaturing reagents, the viral RNA is not secured. Therefore it is essential, that samples are immediately flash frozen subsequent to the harvesting by using liquid nitrogen and are stored at -80°C. Viral RNA contained in such deep frozen samples is stable for months. Viral RNA purification should be processed as soon as possible. Samples can also be stored in the dissolved Lysis Buffer in the Extraction Tube L for 1 h at room temperature, overnight at 4°C, and for long term storage at –80°C. Storage under deep frozen conditions is recommended.

Biopsy material/ tissue: Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. Use of poor quality starting material also leads to reduced length and influences yield of purified DNA. The amount of purified DNA from max.10 mg tissue sample depends on the nature of starting material. The thawing process could proceed

Cultivated bacterial: Bacteria have to be pelleted after cultivation. Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.

Processing of bacterial samples: The kit was validated with *Bacillus subtilis* spiked cell-free medium. To perform a quantitative extraction of bacterial DNA from gram positive Bacteria addition of Lysozyme is needed. Please add 5 µl of a 10 mg/ ml Lysozyme-solution per 200 µl sample volume to the primary tube before starting the assay.

Urine: The bacteria must be pelleted while the supernatant is completely removed (urea contaminations can inhibit PCR reactions). Best results are obtained with fresh peletted material or bacteria pellets that has been immediately frozen and stored at –20°C or –80°C. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size. The amount of purified DNA from max. 15 - 50 ml urine depends on the included bacteria titre.

Stool samples: Best results are obtained with fresh material. Stool samples contain DNases and RNases which realize quickly DNA and RNA digestion and degradation. The sample may be stored at -80°C.

Cell culture supernatants: Best results are obtained with fresh material or material that has been immediately frozen and stored at –20°C or –80°C after winning of the cell culture supernatant. Repeated freezing and thawing of stored samples can influence the sensitivity.

Amniotic fluid*:* The protocol works with fresh amniotic fluid and amniotic fluid stored at 4°C. Best results are obtained using freshly collected samples. The sample can be stored at 4 °C for at least 3 month. For long term storage the samples can be stored at -20 °C.(Avoid multiple thawing and freezing cycles of the sample(s) before isolating the DNA/ RNA.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Preparation of starting materials

1. Extraction of NA from blood, serum, plasma, cell free body fluids, urine, liquor, transport media

This type of sample can be processed directly without any pre-preparations.

Please keep in mind that the first step in the equipment is premixing of samples. Samples have to be at least “pipetable”, mean the presence of clumps and other solid materials leads to clots and prevents a normal workflow of the process. We recommend strictly controlling samples for coagulation by mixing several times overhead before usage on the instrument. For blood take care, that it is well stored and stabilized with EDTA or Citrate. Please use Protocol 1 blood, for all other materials use Protocol 2.

2. Extraction of NA from rinsed liquid from swab samples

a) the sample will also be used for cultivation

Cut off the relevant part of the swab and transfer it into an RNase/ DNAse-free 2 ml tube. Add 400 µl physiological saline solutions to the swab and vortex intensely for 2-3 min and incubate for 10 min at RT. Take an aliquot for cultivation. Transfer 200 µl of the rinsed liquid into a 2.0 ml Safe-Lock-Tube and follow step 1 for NA extraction from bacteria or follow step 2 for NA extractions from virus in protocol 2.

optional: *If bacterial DNA is processed 20 µl Lysozyme can be added to 200 µl sample, follow the instructions of protocol 2.*

Note: *This does not include any warranty for efficiency of the used cultivation method.*

b) the sample will not be used for cultivation

Cut off the relevant part of the swab and transfer this part into an RNase- and DNAse-free 2 ml tube. Add 400 µl RNase-free water to the swab and vortex intensely for 3 min. Optional, incubate for 3 min at 95°C. Transfer 200 µl of the rinsed liquid into a 2.0 ml Safe-Lock-Tube and follow step 1 for NA extraction from bacteria or follow step 2 for NA extractions from virus in protocol 2.

optional: *If bacterial DNA is processed 20 µl Lysozyme can be added to 200 µl sample, follow the instructions of protocol 2.*

3. Extraction of NA from sputum

Transfer 200 µl from the sputum sample into an RNase/DNAse-free tube and add 200 µl NAC Buffer (order number: 1033221100) (ratio sample to buffer must be 1:1) or add 400 µl saturated Acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:2). Incubate the mixture for 10 min at 95°C under shaking on the thermomixer to reduce the viscosity and transfer 200 µl from the mixture into a 2.0 ml Safe-Lock-Tube and follow step 1 for NA extraction from bacteria or follow step 2 for NA extractions from virus in protocol 2.

4. Extraction of NA from slimy tracheal secretes or BAL

Non viscous samples: For isolation of bacterial DNA transfer 1 ml of tracheal secret or BAL into a RNase/DNAse-free tube and centrifuge at 11.100 x g (11.000 rpm) for 3 min. Discard / decant the supernatant without disturbing the bacterial pellet: Resuspend the bacterial pellet in 200 µl distilled water or RNAse free water and transfer the sample into a 2.0 ml Safe-Lock-Tube and follow step 1 for NA extraction from bacteria or follow step 2 in protocol 2. For viral NA use the origin sample and follow step 2 in protocol 2

optional: *If bacterial DNA is processed 20 µl Lysozyme can be added to 200 µl sample, follow the instructions of protocol 2.*

Viscous sample: Transfer e.g. 1 ml of tracheal secretes or BAL into a RNase- and DNAse-free tube and add 1 ml NAC Buffer (order number: 1033221100, ratio sample to buffer must be 1:1) or 2 ml saturated Acetylcysteine (ACC) solution to the sample (ratio sample to buffer must be 1:2)

Incubate the mixture for 10 min at 95°C to reduce the viscosity and centrifuge at 11.100 x g (11.000 rpm) for 3 min. For isolation of viral NA take a 200 µl aliquote into a 2.0 ml Safe-Lock-Tube and follow step 2 in protocol 2.

For isolation of bacterial DNA discard the supernatant without disturbing the bacterial pellet directly. Resuspend the bacterial pellet in 200 µl distilled water or RNase free water and transfer it into a 2.0 ml Safe-Lock-Tube and follow step 1 in protocol 2.

5. Extraction of viral NA from supernatant of stool suspension

Transfer 100 µl stool sample into a 2 ml tube and add 900 µl RNase-free Water. Vortex the sample for 30 s followed by a 1 min centrifugation step at 12.000 x *g* (13.000 rpm).

Transfer 200 µl virus containing supernatant into a 2.0 ml Safe-Lock-Tube (prevent the aspiration of swimming particles) and follow step 2 in protocol 2.

6. Extraction of bacterial NA from supernatant of stool suspension

Transfer 100 µl stool sample into a 2 ml tube and add 300 µl RNase-free Water. Vortex the sample for 30 s followed by a 30 s centrifugation step at 3.000 rpm. (1.000 x *g*)

Transfer 200 µl of the bacteria containing supernatant into a 2.0 ml Safe-Lock-Tube (prevent the aspiration of swimming particles) and follow step 1 in protocol 2.

7. Extraction of NA from Biopsy material/ tissue

Transfer 1 - 10 mg from the tissue biopsy sample into tube and add 200 µl distilled water, 200 µl Lysis Buffer HLT, 20 µl Carrier RNA and 20 µl Proteinase K to the sample If genomic DNA shall be prepared, the addition of Carrier RNA to the Master Mix or to the sample is optional.

8. Extraction from bacterial culture:

Transfer 1ml of the bacterial culture into a 2.0 ml Safe-Lock-Tube (not provided). Centrifuge the overnight culture for 2 min at 8000 rpm and remove completely the supernatant. Resuspend the bacteria pellet in 200 µl PBS Buffer (not provided) and follow step 1 in protocol 2.

9. Extraction from amniotic fluid

Open the infected egg after cultivation for 5-7 days at 37°C (depending on the virus). Transfer 200 µl of the allantois liquid into a 2.0 ml Safe-Lock-Tube and follow step 2 in protocol 2.

10. Extractions from supernatant from organ abrasions

Transfer 100-200 mg tissue material into a 2.0 ml Safe-Lock-Tube (not provided). Add 800 µl physiological saline solutions to the material and disrupt the material by using a mill or shredder. (e.g. Fastprep from MP Biomedical). After the homogenization process, wait 10 min for sedimentation of the bigger particles. Transfer 200 µl of the cleared supernatant into a 2.0 ml Safe-Lock-Tube and follow step 1 for NA extraction from bacteria or follow step 2 for NA extractions from virus in protocol 2.

Lysis procedures

For easier handling we recommend to prepare a Master Mix only for the needed amount of samples consisting of Lysis Buffer HLT, Proteinase K and if required Carrier RNA. When preparing the **Master Mix** it is recommended to use a volume of 5 % greater than that required. The **Master Mix** is stable for at least 2h at RT.

Preparation of a Master Mix

Number of samples	Amount of **Lysis Buffer HLT**	Amount of **Carrier RNA**	Amount of **Proteinase K**
	200 µl / sample	20 µl / sample	20 µl / sample
6	1.3 ml	130 µl	130 µl
8	1.7 ml	170 µl	170 µl
10	2.1 ml	210 µl	210 µl
12	2.6 ml	260 µl	260 µl
16	3.4 ml	340 µl	340 µl
20	4.2 ml	420 µl	420 µl
24	5.0 ml	500 µl	500 µl
32	6.7 ml	670 µl	670 µl
40	8.4 ml	840 µl	840 µl
48	10.0 ml	1000 µl	1000 µl

Extraction control

Extraction control DNA or RNA must be combined with the provided Carrier RNA in one mixture. The vials with Carrier RNA contain 240 µl, 1.2 ml or 2.0 ml stock solutions depending on the package size.

Add the respective amount of Extraction Control Nucleic Acid to the Carrier RNA, if it is in a bigger volume (> 25%) you may replace the according amount of RNAse free water.

Notes:

If you only have indication of amount per reaction, please calculate by using eluate and template volume.

If the extraction control is stable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs (e.g., armored RNA), it can alternatively be added to the sample shortly before beginning sample preparation.

If the extraction control is naked DNA or RNA, it is unstable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs and must not be added directly to the samples. In carrier RNA, an extraction control stabilized.

Refer to the manufacturer's instructions to determine the optimal amount of extraction control for specific downstream applications. Using an amount other than that recommended may lead to wrong quantification results.

Instructions

The following notes are valid for all protocols:

Note: *The DNA/ RNA can also be eluted with a lower (but not lower than 40 µl) or a higher volume of Elution Buffer M (depends on the expected yield or needed concentration of the DNA/ RNA).The eluate can contain viral DNA, viral RNA, bacterial or genomic DNA.*

Important *After extraction place the Elution Tube on ice. For long time storage place the nucleic acids at –20°C or –80°C.*

Note: *The centrifugation steps were made with the* ***Centrifuge 5415 D from Eppendorf.*** *The indicated* ***rpm amounts*** *are referring to this centrifuge.*

Scheme for the Isolation of genomic DNA from blood

genomic DNA

Please read protocols prior the start of the preparation carefully

---

Transfer 100 µl of blood-sample into a 2.0 ml Safe-Lock-Tube and add 100 µl Elution Buffer M.

Add **200 µl Lysis Buffer HLT** and **20 µl Proteinase K,** vortex vigorously,

Incubate for **15 min at 56°C** while continuously shaking

Add **260 µl Binding Solution** and mix by pipetting up and down four times or vortexing

Incubate the sample **at room temperature for 5 minutes**

Take a **RTA Spin Filter Set.**
Transfer lysate onto the RTA Spin Filter
Centrifuge for **1 min at 11.100 x g (11.000 rpm)**
Discard the filtrate and the RTA Receiver Tube

Transfer the RTA Spin Filter in a new RTA Receiver Tube
Add **600 µl Wash Buffer HLT**
Centrifuge for **1 min at 11.100 x g (11.000 rpm)**
Discard the filtrate and the RTA Receiver Tube

Place the RTA Spin Filter into a new RTA Receiver Tube
Add **700 µl Wash Buffer**
Centrifuge for **1 min at 11.100 x g (11.000 rpm)** Discard the filtrate and put the RTA Spin Filter back into the used RTA Receiver Tube.

repeat this washing step once

centrifuge for **5 min at 11.100 x g (11.000 rpm)**. Discard the RTA Receiver Tube with filtrate

Place the RTA Spin Filter into a 1.5 ml Receiver Tube
Add 100 - 200 µl of **Elution Buffer M** (preheated to 56°C)

Incubate for 1 min at room temperature

Centrifuge for **1 min at 11.100 x g (11.000 rpm)**
Discard the RTA Spin Filter
Close the 1.5 ml Receiver Tube and store the DNA sample at 4 °C, for long term storage at -20°C to -80°C.

Protocol 1: Isolation of genomic DNA from blood

Please read the protocols carefully prior to the start of the preparation procedure!

Important Note: *Prewarm the needed amount of **Elution Buffer M to 56°C** for the final elution step. The protocol has been optimized for the isolation of total nucleic acids from 100 µl blood or other body fluids of 200 µl. For samples which have a smaller volume than 200 µl please fill up to a total volume of 200 µl with Elution Buffer M.*

1. **Sample Lysis:**

   In a 2 ml Safe-Lock-Tube mix 100 µl of the blood sample with 100 µl **Elution Buffer M**, add 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K**. Vortex the sample vigorously for 10 seconds. Place the Tubes into a thermomixer and incubate under continuously shaking for 15 minutes at 56°C.

Attention: *by using animal blood the amount of starting material can vary between 20 and 100 µl. Before starting using the kit-make please a dilution series and detect the optimal amount.*

2. **Binding of the DNA and RNA**:

   Add 260 µl **Binding Solution** to the lysed sample and mix the sample completely by pipetting up and down or by vortexing. Incubate the sample at room temperature for 5 minutes. Transfer the sample into the RTA Spin Filter put in a RTA Receiver Tube. Close the cap and centrifuge for 1 minute at 11.100 x g (11.000 rpm). Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

3. **First Washing of the RTA Spin Filter:**

   Add 600 µl **Wash Buffer HLT** to the RTA Spin Filter and centrifuge at 11.100 x g (11.000 rpm) for 1 min. Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

4. **Second Washing of the RTA Spin Filter:**

   Add 700 µl **Wash Buffer** to the RTA Spin Filter and centrifuge at 11.100 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back into the used RTA Receiver Tube.

5. **Repeat this washing step once.**

6. **Ethanol removal:**

   Remove the residual ethanol by final centrifugation for 5 min at 11.100 x g (11.000 rpm). Discard the RTA Receiver Tube with filtrate.

7. **Elution of the DNA/ RNA:**

   Place the RTA Spin Filter into a 1.5 ml Elution Tube and add 100 - 200 µl of the **Elution Buffer M** (prewarmed to 56°C) directly onto the RTA Spin Filter surface.

8. Incubate for 1 min at room temperature and centrifuge at 11.100 x g (11.000 rpm) for 1 minute. Discard the RTA Spin Filter. Close the 1.5 ml Receiver Tube and store the DNA sample at 4 °C, for long term storage at -20°C to -80°C.

Scheme for the simultaneous isolation of pathogen DNA and RNA from all liquid samples

Pathogen NA

Please read protocols prior the start of the preparation carefully

---

Transfer 200 µl of origin or pretreated sample into a 2.0 ml Safe-Lock-Tube.
For samples which have a smaller volume than 200 µl please adjust to a total volume of 200 µl with Elution Buffer M.

Only for isolation of bacterial DNA

Add **20 µl Lysozyme** and **20 µl Carrier RNA** and mix vigorously by vortexing.
Incubate for **10 min at 37°C**
Add **200 µl Lysis Buffer HLT** and **20 µl Proteinase K,** vortex vigorously,

Only for isolation of viral DNA and RNA:

Add **200 µl Lysis Buffer HLT, 20 µl Carrier RNA** and **20 µl Proteinase K,** vortex vigorously,

Note: *If you handle more than 5 preps at the same time we suggest preparing a Master Mix (as described at point Lysis Procedure, page 14). Add 240 µl Master Mix to each sample instead of Carrier RNA, Lysis Buffer HLT and Proteinase K.*

For all:

Incubate for **10 min at 65°C** and then for **10 min at 95°C** while continuously shaking

Add **260 µl Binding Solution** and mix by pipetting up and down four times or vortexing

Incubate the sample at room temperature for 5 minutes

Take a **RTA Spin Filter System.**
Transfer lysate onto the RTA Spin Filter
Centrifuge for **1 min at 11.100 x g (11.000 rpm)**
Discard the filtrate and the RTA Receiver Tube

Transfer the RTA Spin Filter in a new RTA Receiver Tube
Add **600 µl Wash Buffer HLT**
Centrifuge for **1 min at 11.100 x g (11.000 rpm)**
Discard the filtrate and the Receiver Tube

Place the RTA Spin Filter into a new RTA Receiver Tube
Add **700 µl Wash Buffer**
Centrifuge for **1 min at 11.100 x g (11.000 rpm)** Discard the filtrate and put the RTA Spin Filter back into the used RTA Receiver Tube.

repeat this washing step once
centrifuge for **5 min at 11.100 x g (11.000 rpm)**. Discard the RTA Receiver Tube with filtrate

Place the RTA Spin Filter into a 1.5 ml Receiver Tube
Add 100 - 200 µl of **Elution Buffer M** (preheated to 65°C)
Incubate for 1 min at room temperature
Centrifuge for **1 min at 11.100 x g (11.000 rpm)**
Discard the RTA Spin Filter

Close the 1.5 ml Receiver Tube and store the DNA sample at -20 °C to -80°C °C

Protocol 2: Simultaneous isolation of pathogen DNA and RNA from all liquid samples

Please read the protocols carefully prior to the start of the preparation procedure!

Important Note: *Prewarm the needed amount of **Elution Buffer M to 65°C** for the final elution step. The protocol has been optimized for the isolation of total nucleic acids from body fluids of 200 µl (blood 100 µl). For samples which have a smaller volume than 200 µl please fill up to a total volume of 200 µl with Elution buffer M.*

1a) Sample Lysis for bacterial NA

Note: ***For parallel isolation of bacterial DNA and viral RNA please use Lysis Protoocl 1b)***

Note: ***Bacterial culture****: centrifuge max. 0.5 ml of an overnight culture for 2 min at 8000 rpm and remove completely the supernatant. Resuspend the bacteria pellet in 200 µl PBS Buffer (not provided).*

In a 2 ml Safe-Lock-Tube mix 200 µl of the sample with 20 µl **Lysozym mixture** (10 mg/ml) and 20 µl **Carrier RNA**. Mix vigorously by vortexing. Incubate for 10 min at 37°C. Add 200 µl **Lysis Buffer HLT** and 20 µl **Proteinase K**.

Note: *If you handle more than 5 preps at the same time we suggest preparing a Master Mix (as described at point Lysis Procedure, page 14).*
Add 240 µl Master Mix to each sample instead of Carrier RNA, Lysis Buffer HLT and Proteinase K.

Vortex the sample vigorously for 10 seconds. Place the Tubes into a thermomixer and incubate under continuously shaking for 10 to 15 minutes at 65 °C (and then optional for 10 min at 95°C.)

1b) Sample Lysis for simultaneous isolation of bacterial NA and viral NA

In a 2 ml Safe-Lock-Tube mix 200 µl of the sample with 200 µl **Lysis Buffer HLT**, 20 µl **Carrier RNA** and 20 µl **Proteinase K**.

Note: *If you handle more than 5 preps at the same time we suggest preparing a Master Mix (as described at point Lysis Procedure, page 14).*
Add 240 µl Master Mix to each sample instead of Carrier RNA, Lysis Buffer HLT and Proteinase K.

Vortex the sample vigorously for 10 seconds. Place the Tube into a thermomixer and incubate under continuous shaking for 10 minutes at 65°C and then for 10 minutes at 95°C.

Before you add Lysozym to the mixture, take care that the sample is cooled down to < 40°C.

Add 20 µl Lysozyme (stock 10 mg/ ml, not provided) to the lysed sample and incubate for 10 min under shaking at RT.

1c) Sample Lysis for virus NA:

In a 2 ml Safe-Lock-Tube mix 200 µl of the sample with 200 µl **Lysis Buffer HLT**, 20 µl **Carrier RNA** and 20 µl **Proteinase K**.

Note: *If you handle more than 5 preps at the same time we suggest preparing a Master Mix (as described at point Lysis Procedure, page 14).*
Add 240 µl Master Mix to each sample instead of Carrier RNA, Lysis Buffer HLT and Proteinase K.

Vortex the sample vigorously for 10 seconds. Place the Tube into a thermomixer and incubate under continuous shaking for 10 minutes at 65°C and then for 10 minutes at 95°C.

Note stool samples*: These samples have to be diluted 1:10 with RNAse free water. Vortex the sample for 30 sec. Centrifuge the sample for 1 min at 12.000 rpm and transfer the supernatant in a new tube (not provided).*

2. Binding of the DNA and RNA:

Add 260 µl **Binding Solution** to the lysed sample and mix the sample completely by pipetting up and down or by vortexing. Incubate the sample at room temperature for 5 minutes. Transfer the sample into the RTA Spin Filter put into a RTA Receiver Tube. Close the cap and centrifuge for 1 minute at 11.100 x g (11.000 rpm). Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

3. First washing of the RTA Spin Filter:

Add 600 µl **Wash Buffer HLT** to the RTA Spin Filter and centrifuge at 11.100 x g (11.000 rpm) for 1 min. Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube.

4. Second washing of the RTA Spin Filter:

Add 700 µl Wash Buffer to the RTA Spin Filter and centrifuge at 11.100 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back into the used RTA Receiver Tube.

Repeat this washing step once.

5. Ethanol removal:

Remove the residual ethanol by final centrifugation for 5 min at 11.100 x g (11.000 rpm). Discard the RTA Receiver Tube with filtrate.

6. Elution of the DNA/ RNA:

Place the RTA Spin Filter into a 1.5 ml Elution Tube and add 100 - 200 µl of the **Elution Buffer M** (prewarmed to 65°C) directly onto the RTA Spin Filter surface.

Incubate for 1 min at RT and centrifuge at 11.100 x g (11.000 rpm) for 1 minute. Discard the RTA Spin Filter. Close the 1.5 ml Receiver Tube and store the sample at -20 °C to -80°C.

Additional protocol: Simultaneous isolation of nucleic acids* from tissue biopsies

Please read the protocols carefully prior to the start of the preparation procedure!

Important Note: *Prewarm the needed amount of **Elution Buffer M** to 65°C for the final elution step.*

Switch on heating blocks (e.g. thermomixer) to 65°C and 95 °C.

1. Sample Lysis

Transfer 1 - 10 mg from the tissue biopsy sample into a 2.0 ml Safe-Lock-Tube and add 200 µl distilled water or PBS, 200 µl **Lysis Buffer HLT**, 20 µl Carrier RNA and 20 µl Proteinase K to each sample.

Note: *If you handle more than 5 preps at the same time we suggest preparing a Master Mix (as described at point Lysis Procedure, page 14).*
Add 240 µl Master Mix to each sample instead of Carrier RNA, Lysis Buffer HLT and Proteinase K.

The addition of Carrier RNA to the sample is here optional. Place the Tube into a thermomixer and incubate under continuous shaking for **10 minutes at 65°C** and then for **10 minutes at 95°C.**

Lysis times may be increased if the lysis is not completely.

For Bacterial DNA

Before you add Lysozym to the mixture, take care that the sample is cooled down to < 40°C.

Add 20 µl Lysozyme (stock 10 mg/ ml, not provided) to the lysed sample and incubate for 10 min under shaking at RT.

Important: *A longer lysis time could be reduce the final yield and quality of some viral RNA species.*

After lysis centrifuge the sample at max. speed for 1 minute to spin down unlysed material. Transfer the cleared supernatant completely into a 1.5 ml reaction tube (not provided).

2. Binding of the DNA and RNA

Add 260 µl **Binding Solution** to the 1.5 ml reaction tube and mix the sample completely by pipetting up and down or by vortexing. Transfer the sample into the RTA Spin Filter put into a RTA Receiver Tube. Close the cap and centrifuge for 2 minutes at 11.000 x g (11.000 rpm). Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new Receiver Tube.

3. First Washing of the RTA Spin Filter

Add 600 µl **Wash Buffer HLT** to the RTA Spin Filter and centrifuges at 11.000 x g (11.000 rpm) for 1 min. Discard the Receiver Tube with filtrate and place the RTA Spin Filter into a new Receiver Tube.

4. Second Washing of the RTA Spin Filter

Add 700 µl **Wash Buffer** to the RTA Spin Filter and centrifuge at 11.000 x g (11.000 rpm) for 1 min.

*) nucleic acids: include genomic DNA, bacterial DNA, viral DNA and viral RNA

5. Ethanol removal

Discard the RTA Receiver Tube with filtrate and place the RTA Spin Filter into a new RTA Receiver Tube. Remove the residual ethanol by final centrifugation for 4 min at maximum speed. Discard the RTA Receiver Tube with filtrate.

6. Elution of the DNA/ RNA

Place the RTA Spin Filter into a 1.5 ml Elution Tube**.** Add 60 µl of the **Elution Buffer M** (prewarmed to 65°C) directly onto the RTA Spin Filter surface. Incubate for 3 minutes at RT and centrifuge at 11.000 x g (11.000 rpm) for 1 minute.

Troubleshooting

Problem	Probable cause	Comments and suggestions
Common errors	**Proteinase K** volume/concentration too low reagent / buffer volume too low	make sure that you have resuspended the lyophilized **Proteinase K** with the appropriate volume of water before use
low concentration of extracted DNA/RNA	no/ too much isopropanol added to **Wash Buffer HLT**	ensure that the **Wash Buffer HLT** has been filled up with isopropanol properly as indicated in Tab. 1
	no/ too much ethanol added to **Wash Buffers**	ensure that the **Wash Buffer** has been filled up with ethanol properly as indicated in Tab. 1
	incorrect storage of starting material	ensure that the storage of starting material was correct avoid multiple freezing and thawing cycles of the material
degraded or sheared DNA/RNA	old material	ensure that the starting material is fresh or stored under appropriate condition (for long time storage at –20°C)! avoid multiple thawing and freezing cycles of the material old material often contains degraded DNA/RNA
	combination of reagents from different kits	please make sure that only reagents belonging to one kit type are used. a combination of reagents belonging to different kit types will not work

Appendix

General notes on handling DNA

Starting material
This kit is designed for extraction of DNA from saliva and swabs. These materials show big variation in DNA contents. The purification of some apoptotic DNA is normal.

Nature of DNA
The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, and long-template PCR.

Storage of DNA
A working stock of DNA can be stored at 2 – 4˚C for several weeks. For long term storage DNA should be stored at -20˚C, but storing at – 20°C can cause shearing, particularly if the DNA is exposed to repeated freeze-thaw cycles.

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA
Avoid over drying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution.
Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

General notes on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases has to be reduced as much as possible Avoid handling bacterial cultures, cell cultures, or other biological sources of RNases in the same lab where the RNA purification is to be carried out.

All glassware should be treated before use to ensure that it is RNase free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240 °C for four or more hours before use. Autoclaving alone will not completely inactivate many RNases. Oven baking will both inactivate RNases and ensure that no other nucleic acids (such as Plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware must stand 12 hours at 37 °C and then be autoclaved or heated to 100 °C for 15 minutes to remove residual DEPC.

- **Electrophoresis tanks** should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase free water, and then rinsed with ethanol and allowed to dry.
- **Non-disposable plasticware** should be treated before use to ensure that it is RNase free. plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All **buffers** must be prepared from DEPC-treated RNase free ddH_2O.
- Change gloves frequently and keep tubes closed.
- Reduce the preparation time as much as possible.
- **Use only sterile, disposable polypropylene tubes** throughout the procedure. (These tubes are generally RNase free.)
- Keep isolated RNA on ice.

This kit should only be used by personnel trained in laboratory practice.

Storage of RNA
Purified RNA can be stored –80°C and is stable for months and years e.g. precipitated and stored in 70% ethanol.

Quantification
Quantification of DNA and RNA from this assay must be done by means of qPCR or Reverse Transcriptase qPCR. All other methods will be disturbed by the included Carrier Nucleic Acids as well as DNA or RNA which is co-purified.

Ordering information

Product	Package Size	Catalogue No.
Invisorb® Spin Universal Kit Kit	50 preparations	1050100200
Invisorb® Spin Universal Kit Kit	250 preparations	1050100300

Related Products	Package Size	Catalogue No.
InviMag® Universal Kit /KF96	1 x 96 preparations	7450300100
InviMag® Universal Kit /KF96	5 x 96 preparations	7450300200
InviMag® Universal Kit / STARlet	4 x 96 preparations	7450330300
InviMag® Universal Kit / STARlet	24 x 96 preparations	7450330400
Invisorb® Universal HTS 96 Kit/ STARlet	4 x 96 preparations	7150330300
Invisorb® Universal HTS 96 Kit/ STARlet	24 x 96 preparations	7150330400
InviMag® Universal Kit/ IG	8 x 12 preps	2450120100
InviMag® Pathogen Kit /KF96	1 x 96 preparations	7450300100
InviMag® Pathogen Kit /KF96	5 x 96 preparations	7450300200
InviMag® Pathogen Kit/ KFmL	15 preparations	2450110200
InviMag® Pathogen Kit/ KFmL	75 preparations	2450110300
RTP® Pathogen Kit	50 preparations	1040500200
RTP® Pathogen Kit	250 preparations	1040500300
RTP® DNA/ RNA Virus Mini Kit	50 preparations	1040100200
RTP® DNA/ RNA Virus Mini Kit	250 preparations	1040100300
RTP® Bacteria DNA Mini Kit	50 preparations	1033200200
RTP® Bacteria DNA Mini Kit	250 preparations	1033200300
Invisorb® Virus RNA HTS 96 Kit/ X	4 x 96 preparations	7143310300
Invisorb® Virus RNA HTS 96 Kit/ X	24 x 96 preparations	7143310400
Invisorb® Virus DNA HTS 96 Kit/ X	4 x 96 preparations	7142310300
Invisorb® Virus DNA HTS 96 Kit/ X	4 x 96 preparations	7142310400
InviMag® Virus RNA Mini Kit/ KF 96	1 x 96 preparations	7443300100
InviMag® Virus RNA Mini Kit/ KF 96	5 x 96 preparations	7443300200
InviMag® Virus DNA Mini Kit/ KF 96	1 x 96 preparations	7442300100
InviMag® Virus DNA Mini Kit/ KF 96	5 x 96 preparations	7442300200
InviMag® Virus DNA/ RNA Mini Kit/ KFmL	15 preparations	2441150100
InviMag® Virus DNA/ RNA Mini Kit/ KFmL	75 preparations	2441150200
InviMag® Virus DNA/ RNA Mini Kit	50 preparations	1440100200
InviMag® Virus DNA/ RNA Mini Kit	250 preparations	1440100300
InviMag® Bacteria DNA Mini Kit/ KFmL	15 preparations	2433150100
InviMag® Bacteria DNA Mini Kit/ KFmL	75 preparations	2433150200

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order Nr. 6752

Applichem
2-Propanol
Order Nr. A3928

Sigma
2-Propanol
Order Nr. 59304-1L-F

Possible suppliers for centrifuges:

Eppendorf AG
22331 Hamburg, Germany
Tel.: +49 (0) 40 53801 0
Fax: +49 (0) 40 53801 556
E-Mail: eppendorf@eppendorf.com
Internet: www.eppendorf.com

SIGMA Laborzentrifugen GmbH
37507 Osterode am Harz, Germany
Tel.: +49-5522-5007-0
Fax: +49-5522-5007-12
E-Mail: info@sigma-zentrifugen.de
Internet: www.sigma-zentrifugen.de

stratec molecular

STRATEC Molecular GmbH
Robert-Rössle-Str. 10
13125 Berlin, Germany

Phone: +49 30 94 89 29 01
Fax: +49 30 94 89 29 09
E-mail: info.berlin@stratec.com

www.stratec.com

1A8k/08/2014